CLAIMS

We claim:

1. A composition comprising a polymerizing agent including at least one molecular and/or atomic tag located at or near, associated with or covalently bonded to a site on the polymerizing agent, where a detectable property of the tag undergoes a change before, during and/or after monomer incorporation.

2. The composition of claim 1, wherein the detectable property has a first value when the polymerizing agent is in a first state and a second value when the polymerase is in a second state, and where the polymerizing agent changes from the first state to the second state and back again during each monomer incorporation.

3. The composition of claim 2, wherein the polymerizing agent is a polymerase or reverse transcriptase.

4. The composition of claim 3, wherein the polymerase is selected from the group consisting of *Taq* DNA polymerase I, T7 DNA polymerase, Sequenase, and the Klenow fragment from *E. coli* DNA polymerase I.

5. The composition of claim 3, wherein the reverse transcriptase comprises HIV-1 reverse transcriptase.

6. The composition of claim 3, wherein the polymerase comprises *Taq* DNA polymerase I having a tag attached at a site selected from the group consisting of 513-518, 643, 647, 649 and 653-661 and mixtures or combinations thereof of the *Taq* polymerase, where the tag comprises a fluorescent molecule.

7. A composition comprising a polymerase or reverse transcriptase including at least one molecular and/or atomic tag located at or near, associated with or covalently bonded to a site on the polymerase, where a detectable property has a first value when the polymerase is in a first state and a second value when the polymerase is in a second state during monomer incorporation, and where the polymerizing agent changes from the first state to the second state and back again during each

monomer incorporation.

8. The composition of claim 7, wherein the polymerase is selected from the group consisting of *Taq* DNA polymerase I, T7 DNA polymerase, Sequenase, and the Klenow fragment from *E. coli* DNA polymerase I.

9. The composition of claim 7, wherein the reverse transcriptase comprises HIV-1 reverse transcriptase.

10. A composition comprising a polymerizing agent including a molecular and/or atomic tag associated with or covalently bonded to a site on the polymerase and a monomer including a molecular and/or atomic tag, where at least one of the tags has a detectable property that undergoes a change before, during and/or after monomer incorporation due to an interaction between the polymerizing agent tag and the monomer tag.

11. The composition of claim 10, wherein the change in the detectable property results from a change in the conformation of the polymerase from a first conformational state to a second conformational state and back again during each monomer incorporation.

12. The composition of claim 10, wherein the detectable property has a first detection propensity when the polymerase is in the first conformational state and a second detection propensity when the polymerase is in the a second conformational state.

13. The composition of claim 12, wherein the polymerizing agent is a polymerase or reverse transcriptase.

14. The composition of claim 13, wherein the polymerase is selected from the group consisting of *Taq* DNA polymerase I, T7 DNA polymerase, Sequenase, and the Klenow fragment from *E. coli* DNA polymerase I.

15. The composition of claim 13, wherein the reverse transcriptase comprises HIV-1 reverse transcriptase.

16. The composition of claim 12, wherein the monomer comprise a dNTP and the tag is covalently bonded to the β or γ phosphate group.

17. The composition of claim 10, wherein the tag comprises a fluorescent tag and the detectable property comprises an intensity and/or frequency of emitted light.

18. The composition of claim 16, wherein the detectable property is substantially active when the polymerase is in the first conformational state and substantially inactive when the polymerase is in the second conformational state or substantially inactive when the polymerase is in the first conformational state and substantially active when the polymerase is in the second conformational state.

19. The composition of claim 14, wherein the polymerase comprises *Taq* DNA polymerase I having a tag attached at a site selected from the group consisting of 513-518, 643, 647, 649 and 653-661 and mixtures or combinations thereof of the *Taq* polymerase, where the tag comprises a fluorescent molecule.

20. A composition comprising a polymerase or reverse transcriptase including a pair of tags located at or near, associated with or covalently bonded to a site of the polymerase, where a detectable property of at least one of the tags undergoes a change before, during and/or after monomer incorporation.

21. The composition of claim 20, wherein the detectable property has a first value when the polymerase is in a first state and a second value when the polymerase is in a second state, and where the polymerizing agent changes from the first state to the second state and back again during each monomer incorporation.

22. The composition of claim 21, wherein the polymerase is selected from the group consisting of *Taq* DNA polymerase I, T7 DNA polymerase, Sequenase, and the Klenow fragment from *E. coli* DNA polymerase I.

23. The composition of claim 21, wherein the reverse transcriptase comprises HIV-1 reverse transcriptase.

24. The composition of claim 22, wherein the polymerase comprises *Taq* DNA polymerase I having a tag attached at a site selected from the group consisting of 513-518, 643, 647, 649 and 653-661 and mixtures or combinations thereof of the *Taq* polymerase, where the tag comprises a fluorescent molecule.

25. A single molecule sequencing apparatus comprising a substrate having a first chamber in which at least one tagged polymerase is confined therein and a second chamber including tagged dNTPs and a channel interconnecting the chambers, where a detectable property of at least one tag undergoes a detectable change during a monomer incorporation cycle.

26. The apparatus of claims 25, further comprising a plurality of monomer chambers, one for each tagged dNTP.

27. A mutant Taq polymerase comprising native Taq polymerase with a cysteine residue replacement at a site selected from the group consisting of 513-518, 643, 647, 649 and 653-661 and mixtures or combinations thereof.

28. The polymerase of claim 27, wherein the cysteine residue includes a tag covalently bonded thereto through the SH group.

29. A system for retrieving stored information comprising:

a unknown nucleotide sequence representing a data stream;

a single-molecule sequencer including a polymerase having a tag associated therewith and monomers for the polymerase, each monomer having a tag associated therewith;

an excitation source adapted to excite the at least one of the tags; and

a detector adapted to detect a response from at least one of the tag,

where the response changes during polymerization of a complementary sequence and the changes in response represent a content of the data stream.

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29. A system for determining sequence information from a single molecule comprising:

a unknown nucleotide sequence;

a single-molecule sequencer comprising a polymerase having a tag associated therewith and monomers for the polymerase, each monomer having a tag associated therewith;

a excitation source adapted to excite at least one of the tags; and

a detector adapted to detect a response from at least one of the tags,

where the response changes during polymerization of a complementary sequence and the changes in the response represent the identity of each nucleotide in the unknown sequence.

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30. A method for sequencing a molecular sequence comprising:

supplying an unknown sequence of nucleotides or nucleotide analogs to a single-molecule sequencer comprising a polymerase having a fluorescent donor covalently attached thereto and monomers for the polymerase, each monomer having a unique fluorescent acceptor covalently bonded thereto;

exciting the fluorescent donor with a light from an excitation light source;

detecting emitted fluorescent light from the acceptor during a monomer incorporation cycle via a fluorescent light detector, where an intensity and/or frequency of the emitted light for the acceptors changes during each monomer incorporation cycle; and

converting the changes into an identity of each nucleotide or nucleotide analog in the unknown sequene.

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31. A method of sequencing an individual nucleic acid molecule or numerous individual molecules in parallel including the steps of:

immobilizing a member of the replication complex comprising a polymerase including a tag attached thereto, a primer or a template sufficiently spaced apart to allow resolution detection of each complex on a solid support;

incubating the replication complex with cooperatively-tagged nucleotides, each nucleotide including a unique tag at its gamma-phosphate, where each nucleotide can be individually detected;

detecting each nucleotide incorporated by the polymerase as the polymerase transitions between its open and closed form, which causes a change in a detectable property of at least one of the tags or as the pyrophosphate group is released by the polymerase; and

relating the changes in the detectable property to the sequence of nucleotides in an unknown

nucleic acid sequence.

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32. A γ-phosphate modified nucleoside comprising γ-phosphate modified dATP, dCTP, dGTP and dTTP.

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33. A primer sequence or portion thereof selected from the group consisting of Sequence 1 through 29.